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Bloodmeal Identification by Direct Enzyme-Linked Immunosorbent Assay (ELISA), Tested on *Anopheles* (Diptera: Culicidae) in Kenya[1,2]

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ABSTRACT A direct enzyme-linked immunosorbent assay (ELISA) was developed for bloodmeal identification of seven hosts. Commercially available reagents are used; the test can be completed in only 4.5 h. Blood meals can be detected up to 32 h after feeding for dried mosquitoes and up to 23 h for frozen mosquitoes. A two-step procedure, using anti-human peroxidase conjugate and antibovine phosphatase conjugate, was developed to test a single mosquito for two hosts in the same microtiter plate well. The assay was applied to *Anopheles gambiae* Giles s. lat. and *A. funestus* Giles collected inside huts in western Kenya; 94% of 4,338 blood meals were identified as either human (88%), cow (4%), or mixed human–cow (2%). Additionally, a system was developed whereby a single mosquito could be tested by both the blood meal ELISA and the malaria sporozoite ELISA.

KEY WORDS Insecta, blood meal, enzyme-linked immunosorbent assay, *Anopheles*

BLOODMEAL identification for mosquitoes and other hematophagous Diptera is important in the study of malaria and other arthropod-borne diseases. Several serological techniques have been used to detect host-specific blood meals (see reviews by Weitz 1956, Tempelis 1975, Washino & Tempelis 1983). Of the available techniques, the precipitin test has been used most commonly. Enzyme-linked immunosorbent assays (ELISA) have been developed for bloodmeal identification and have proven useful for field studies (Edrissian & Hafizi 1980, Burkot et al. 1981, Edrissian & Hafizi 1982, Lindqvist et al. 1982, Lombardi & Esposito 1983, Linthicum et al. 1985, Service et al. 1986).

Two basic ELISA procedures have been used for bloodmeal identification. In the indirect ELISA, also referred to as the "sandwich technique," host-specific antisera are incubated in 96-well microtiter plates. Homologous immunoglobulins from the bloodmeal sample are captured by anti-IgG on the coated plate. Following a washing step to remove nonantigenic material, specific reactions are detected by applying an enzyme-conjugate of the antibody that is specific to the host in which the antiserum was produced, and the appropriate substrate to produce a color reaction. In the direct ELISA, the bloodmeal sample is incubated directly in the microtiter plate well. The direct ELISA uses a host-specific antibody–enzyme conjugate to detect homologous IgG in the bloodmeal sample. The primary difference between the two procedures is that the indirect ELISA uses an antiserum to capture a specific IgG, and the direct ELISA uses the antibody–enzyme conjugate alone to bind host-specific IgG in the bloodmeal.

Strategies for using ELISAs for blood meals depend upon study objectives. For example, Edrissian et al. (1985) used a direct ELISA to screen over 5,000 *Anopheles* from Iran for human blood meals. They reported that an experienced technician could easily test over 1,000 samples per week. Burkot & DeFoliart (1982) used an indirect ELISA to identify 16 host sources, including wild animals, in Wisconsin. The indirect ELISA is technically more difficult, because an antiserum must be produced for each host to be tested. The indirect ELISA is most appropriate when information is required for a range of wild hosts. The direct ELISA, as used by Edrissian et al. (1985), would potentially be most useful to investigators desiring information on rates of human feeding. Since antibody–enzyme conjugates are now commercially available for a number of domestic hosts, it should be possible to adapt direct ELISA techniques for integrated human and

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domestic host bloodmeal identification. As ELISAs for malaria sporozoites (Burkot et al. 1984, Wirtz et al. 1985) become incorporated into field programs, parallel bloodmeal determinations by the direct ELISA on the same mosquitoes would yield valuable epidemiological information.

This study describes three techniques—a simple, direct ELISA to screen mosquitoes for human, cow, goat, pig, horse, dog, and chicken blood meals; methods to test blood meals for two different hosts in the same microtiter plate well; and methods whereby the bloodmeal ELISA can be used in conjunction with the ELISA for sporozoites. *Anopheles gambiae* Giles s. lat. and *Anopheles funestus* Giles were collected and tested in Kenya to evaluate the ELISA assays.

Materials and Methods

Direct ELISA. *Mosquito Blood Sources.* Laboratory-reared *Anopheles stephensi* Liston were used for all laboratory experiments. Mosquitoes were fed directly on a human, mice, and hamsters. Membrane-feeding techniques (Rutledge et al. 1964) were used to feed mosquitoes on stored cow blood. Blood-fed mosquitoes were killed by freezing and were held either frozen at −20°C or in a desiccator jar at room temperature (19–23°C).

Preparation of Mosquito Samples. Mosquitoes were prepared individually for testing by trituration in 0.2-ml glass microtissue grinders (Kontes Scientific Glassware, Vineland, N.J.) to which 50 μl 0.01 *M* phosphate buffered saline (PBS), pH 7.4, was added. Samples were then mixed with PBS to desired dilutions and frozen at −20°C until tested.

ELISA Procedure. Direct ELISAs were developed for identification of human, cow, goat, pig, horse, dog, and chicken blood meals. Peroxidase conjugates were obtained from Miles Laboratory, Naperville, Ill.; phosphatase-conjugated goat anti-bovine IgG (H&L) was obtained from Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md. Mosquito triturate (50 μl) was diluted in PBS (1:50) and 50-μl volumes were added to wells of polyvinyl choride, U-shaped, 96-well microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.), which were covered and incubated at room temperature for 3 h. Each well was then washed twice with PBS containing 0.5% Tween 20 (PBS-Tw 20). This was followed by the addition of 50 μl host-specific conjugate (antihost IgG, H&L) diluted 1:2,000 (or 1:250 for bovine) in 0.5% boiled casein containing 0.025% Tween 20. The boiled casein was prepared by dissolving 5 g casein in 100 ml 0.1 *N* NaOH by boiling, adding 900 ml PBS, adjusting pH to 7.4, adding 0.1 g Thimerosal (sodium ethylmercurithiosalicylate) and 0.02 gm phenol red, and storing at 4°C (all reagents from Sigma Co., St. Louis, Mo.). After 1 h, wells were washed three times with PBS-Tween 20, and 100 μl of ABTS (2,2′-azino-di-[3-ethyl benzthiazoline sulfonate]) peroxidase substrate (Kirkegaard & Perry) was added to each well. Absorbance at 414 nm was determined with an ELISA reader 30 min after the addition of substrate. The dark green positive reactions for peroxidase, or dark yellow reactions for phosphatase, may also be determined visually.

Samples were considered positive if absorbance values exceeded the mean plus three times the standard deviation of four negative control, unfed mosquitoes. Positive and negative control samples should be tested on each microtiter plate, as interplate variation for absorbance values of controls can be significant if plates are not read at consistent times following the addition of substrate.

A two-step procedure was developed for determining a second host source in the same microtiter plate well where mosquitoes were screened for human blood. A second conjugate, phosphatase-labeled anti-bovine IgG (1:250 dilution of 0.5 mg/ml stock solution) was added to the peroxidase-labeled antihuman IgG solution. Blood meals were screened first for human IgG by the addition of peroxidase substrate according to the methods described above. After reading absorbance at 30 min, the wells were washed 3 times with PBS-Tween 20, and 100 μl phosphatase substrate (Kirkegaard & Perry) was added to each well. Plates were read after 1 h to determine positive cow reactions.

Sensitivity and Specificity. Sensitivity of the direct ELISA for seven hosts was evaluated by testing homologous serum samples diluted in PBS. Five replicates were tested for each four-fold serum dilution from 1:1,000 to 1:16,384,000. Similar tests were run on human-fed mosquitoes killed 2 h after feeding, ground in PBS, and held either frozen at −20°C or dried in a desiccator and stored at room temperature. Five samples of mosquito triturate (ground in 50 μl PBS) were tested in 4-fold dilutions from 1:50 to 1:25,600.

Specificity was determined by testing heterologous serum in each host-ELISA system, comparing absorbance with values for homologous sera. Secondly, 1:500 dilutions of heterologous sera were added to the conjugate solutions to decrease cross-reactivity.

ELISA Activity versus Bloodmeal Digestion. To determine ELISA sensitivity in relation to bloodmeal digestion, *A. stephensi* were fed on a human, held at 27 ± 2°C, and groups of 10 mosquitoes were killed by freezing at 0, 4, 8, 12, 23, 29, 32, 36, 39, 47, and 50 h after feeding. Five mosquitoes from each time interval were frozen at −20°C and five were held dry in a desiccator for more than 1 wk at room temperature. After grinding in 50 μl PBS, dilutions of mosquito triturate (1:50) were tested by ELISA. Negative controls consisted of the same dilutions of fresh and dry unfed triturated mosquitoes and of PBS alone.

Field samples. Blood-fed *A. gambiae* s. lat. and *A. funestus* were collected by aspiration inside houses of the villages of Saradidi and Kisian in Nyanza Province, Kenya, in the morning from October 1985 to August 1986. Mosquitoes were stored

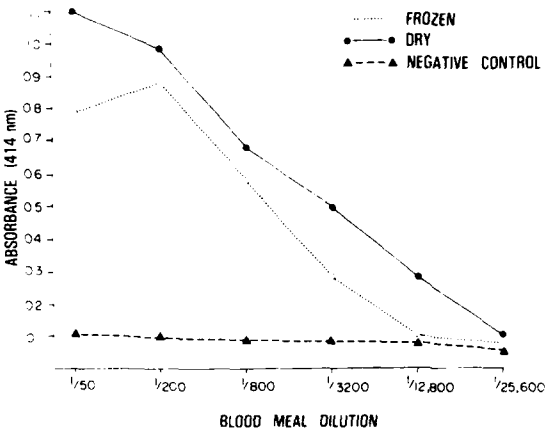

Fig. 1. Mean absorbance values for dilutions in PBS of human blood-fed mosquitoes tested by ELISA to detect human blood meals. Frozen (○) and dried (●) blood-fed mosquitoes (three per group) were ground in 50 μl PBS, and four-fold dilutions were tested. Negative controls consisted of the same dilutions of unfed mosquitoes.

at −20°C within 6 h of collection and were later transported frozen to the Kenya Medical Research Institute (KEMRI) in Nairobi. Freshly engorged mosquitoes were prepared for testing by cutting them transversely at the thorax between the first and third pairs of legs (under a dissecting microscope, 10–20×). The posterior part of the mosquito containing the blood meal was ground in 50 μl PBS and a 1:50 dilution was frozen.

Samples were first screened for human and cow blood using the two-step procedure described above. Nonreacting samples were then tested for goat, pig, horse, dog, and chicken. For each test, 1:500 dilutions of heterologous serum (eight hosts) were added to the conjugate solution to reduce background absorbance. Each plate contained control serum samples (1:500 dilution in PBS) of human, cow, goat, pig, horse, dog, chicken, and cat, and four field-collected male *Anopheles* ground in PBS at the same dilution as test samples.

Compatibility of Bloodmeal and Sporozoite ELISAs. *Comparison of Buffer Systems.* Initial tests showed that mosquitoes ground in PBS were suitable for the bloodmeal ELISA. Comparative bloodmeal ELISAs were run with mosquitoes ground in blocking buffer (BB) (1.0% bovine serum albumin [BSA], 0.5% casein, 0.01% thimerosal, and 0.002% phenol red dissolved in 0.01 *M* PBS pH 7.4), the diluent used in the sporozoite ELISA (Wirtz et al. 1987). Mosquitoes fed on human blood were prepared in the two buffers and tested in two-fold dilutions from 1:200 to 1:3,200.

Preparation of Blood-fed Mosquitoes for Bloodmeal and Sporozoite ELISAs. Because blood-fed mosquitoes ground in BB did not react in the bloodmeal ELISA, an alternative system was tested whereby individual mosquitoes were first ground in 50 μl PBS; a 10-μl aliquot was used for the bloodmeal ELISA, and the remaining 40 μl was used in the sporozoite ELISA. The 10-μl aliquot was diluted to 1:50, 1:100, 1:200, and 1:400 in PBS and tested for human blood by the ELISA procedure. The 40-μl aliquot for the sporozoite ELISA was diluted with 50 μl BB containing 0.5% Nonidet P-40 (Sigma Chemical Co.), then 160 μl BB was added to bring the volume to 250 μl. To determine the sensitivity of this procedure 2,500 salivary gland sporozoites of either *Plasmodium falciparum* or *P. vivax* (volume <5 μl) were obtained from infected mosquitoes (Burkot et al. 1984; Wirtz et al. 1985) and added to the blood-fed mosquito sample or to an unfed mosquito ground in BB (total volume 250 μl, including 50 μl BB and NP-40). This yielded 400 sporozoites per 50 μl. Samples tested by the sporozoite ELISA contained 400, 200, 100, 50, 25, or 12 sporozoites. Negative controls consisted of blood-fed and unfed mosquito triturate in BB.

Results

ELISA Sensitivity and Specificity. The sensitivity of direct ELISA tests for seven hosts was determined by testing homologous serum dilutions (Table 1). Mean absorbance values ranged from 0.25 to 0.61 for dilutions up to 1.0×10^{-3} for the seven host systems and all exceeded PBS control samples at the 1.6×10^{-7} dilution. The ELISA readily detected human blood meals in mosquitoes killed 2 h after feeding, and held either frozen or dry (Fig. 1). Absorbance values decreased with increasing dilutions of the 50-μl ground mosquito samples. The assay detected bloodmeal dilutions

Table 1. Sensitivity of direct ELISAs for seven hosts determined by testing dilutions of homologous sera

Reciprocal serum dilution	Absorbance (414 nm)[a]						
	Human	Cow	Pig	Dog	Goat	Horse	Chicken
1,000	1.69	2.00	1.55	2.00	1.45	2.00	2.00
4,000	2.00	2.00	1.91	2.00	1.64	2.00	2.00
16,000	2.00	2.00	2.00	2.00	1.75	2.00	2.00
64,000	2.00	1.90	1.80	2.00	1.40	2.00	1.42
256,000	1.20	0.92	0.91	1.02	0.62	1.34	0.68
1,024,000	0.55	0.50	0.42	0.43	0.25	0.61	0.32
4,096,000	0.27	0.37	0.21	0.19	0.11	0.33	0.17
16,384,000	0.16	0.30	0.13	0.15	0.08	0.21	0.16

[a] Absorbance values (range 0–2.00) represent the mean of five replicates tested at each dilution.

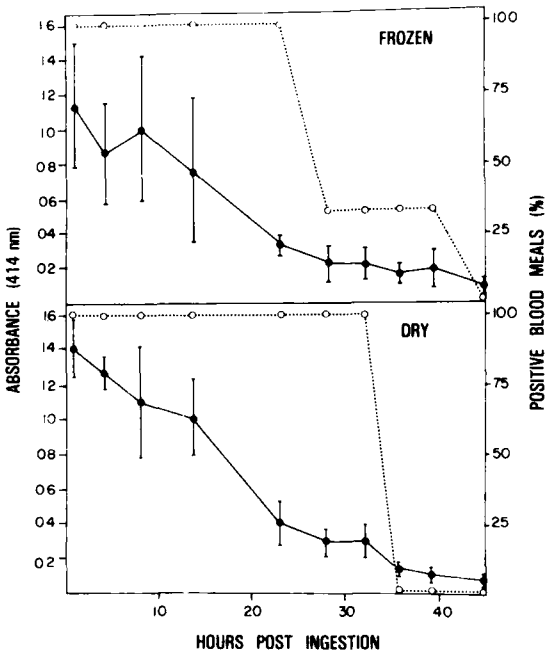

Fig. 2. Human bloodmeal ELISA results for frozen and dried mosquitoes killed at successive time intervals after feeding on human blood. Each point (●) represents the mean (and standard deviation) for three mosquitoes. The percentage of positive-reacting mosquitoes (○) was determined from a cutoff point for negative control, unfed mosquitoes (mean plus 3 × SD).

up to 1:3,200 for frozen mosquitoes and dilutions up to 1:12,800 for dry mosquitoes.

Human blood meals were accurately detected by ELISA up to 32 h after feeding for dried mosquitoes and up to 23 h for frozen mosquitoes (Fig. 2). Mean absorbance values for positive dried mosquitoes were higher than for frozen mosquitoes for all time intervals.

Specificity for the seven host systems was determined by comparing absorbance values for homologous serum with values for seven heterologous sera (Table 2). Tests for human, cow, and chicken were the most specific, with absorbance values less than 0.23 for all heterologous sera. Conjugated antibodies for goat, pig, horse, and dog ELISAs were not specific when used alone. Specificity of all test systems was significantly increased when 1:500 dilutions of all heterologous sera were added to the conjugate solution. Cross-reactivity was reduced to a level where absorbance values were less than 0.11 for heterologous sera.

Additional tests for specificity showed that the antihuman conjugate cross reacted with 1:500 dilutions of sera from three higher primates (*Cercopithecus aethiops*, *C. mitis*, and *Papio anubis*), but no cross-reactivity was seen with sera from the bushbaby (*Galago crassicaudata*) or mice, hamsters, or rabbits. The antihorse conjugate cross-reacted with donkey serum, and the antigoat conjugate cross-reacted with sheep serum.

Bloodmeal ELISA tests on *Anopheles* from Kenya. Of 4,338 blood-fed *Anopheles* tested from two sites in western Kenya, 94.6% of the blood meals were identified by direct ELISAs (Table 3). From the initial screening of *A. gambiae* s. lat. and *A. funestus* blood meals using the human–peroxidase and bovine–phosphatase two-step procedure, 94.4% (4,095/4,338) of the blood meals were identified (88% human, 4% cow, and 2% mixed human and cow). Of the remaining 233 blood meals, ELISA tests for goat, pig, horse, dog, and chicken detected four dog and six goat meals. Additionally, five *Anopheles pharoensis* Theobald blood meals were identified as human, and of two *Anopheles coustani* Laveran blood meals, one was human and the other goat.

Low background absorbance values facilitated accurate bloodmeal identification. Absorbance values for negative control mosquitoes were normally less than 0.10. Values for heterologous sera usually ranged from 0.03 to 0.12, which allowed for cutoff values less than 0.15. Fig. 3 shows the distribution of absorbance values for human- and cow-positive blood meals.

Compatibility of Bloodmeal and Sporozoite ELISAs. Mosquitoes with human blood meals ground in BB (the solution used in the sporozoite ELISA) were not positive when tested in the bloodmeal ELISA. This difficulty was overcome by first grinding the blood-fed mosquito in 50 μl PBS, then using 10 μl for the bloodmeal assay and 40 μl for the sporozoite ELISA. Fig. 4 shows that grinding mosquitoes first in PBS, then using a 40 μl aliquot (with BB added), yielded the same results as mosquitoes prepared in BB as usual for the sporozoite assay. Similar results were observed for *P. falciparum* and *P. vivax* assays. The addition of sporozoites to mosquitoes tested by the bloodmeal ELISA did not affect the assay.

The procedure was used to test 86 *A. gambiae* s. lat. and 164 *A. funestus* collected in Kenya during October 1985. Human blood was detected in 75 of 86 (87.2%) *A. gambiae* and 163 of 164 (99.4%) *A. funestus*. Of those mosquitoes with human blood, 38 of 237 (16%) were positive for *P. falciparum* circumsporozoite antigen.

Discussion

Traditionally, investigators have faced difficulties in identification of blood meals, because this capability existed in only a few laboratories. The direct ELISA described in this study has wide applicability for the identification of human and domestic host blood meals, because the test uses commercially available reagents. Unlike the precipitin test and the indirect ELISA, host-specific antisera are not required. Because of its simplicity, the direct ELISA will be especially useful for determining the human blood index (HBI), an integral parameter for estimating vectorial capacity (Garrett-Jones 1964). The present study shows that the

direct ELISA can also be used for accurate testing of blood meals for domestic hosts.

The selection of a bloodmeal test for field studies will depend upon resources, technical capabilities, and study objectives. While several tests are available for bloodmeal identification, including the new DOT-PAP test (Lombardi & Esposito 1986), the precipitin test has been used most widely (Washino & Tempelis 1983). The advantages of ELISA techniques over precipitin tests are that blood meals can be rapidly identified in microtiter plates, test results are more objective, and sensitivity is about 1,000 times greater (Washino & Tempelis 1983). The direct ELISA is currently most practical for identifying human and domestic hosts, because enzyme-labeled conjugates are not yet commercially available for wild hosts. When screening of a wide variety of host species is required, the indirect ELISA may be preferable, because enzyme-conjugated sera are required only for animal species in which specific host antisera are produced (Burkot et al. 1981).

The direct ELISA compares favorably with indirect methods previously described (Burkot et al. 1981, Lindqvist et al. 1982, Service et al. 1986). The sensitivity of the direct ELISA exceeded 10 ng for seven host systems tested, which is comparable to tests by Lindqvist et al. (1982). Each mosquito sample could be diluted to provide material for a minimum of 50 tests at 1:50 dilutions of the original 50-μl sample. The direct ELISA detected blood meals up to 32 h for dried mosquitoes and up to 23 h for frozen mosquitoes, which is comparable to the improved indirect ELISA methods of Service et al. (1986). This time limit remains one of the disadvantages of ELISAs compared to precipitin tests, which can detect host meals up to 48 h after feeding (Edman & Schmid 1970). The addition of heterologous sera (1:500 dilutions) to the conjugate step improves specificity and is essential for use with goat, pig, horse, and dog tests. Similarly, Service et al. (1986) found it necessary to include heterologous IgG in the conjugate diluent for optimal specificity of the indirect ELISA.

Perhaps the most important improvement in our technique over previous ELISAs is mixing 0.5% boiled casein and Tween 20 with the enzyme. This solution is also used in assays to detect circumsporozoite antibodies in human sera (Wirtz et al., unpublished data). When used with the enzyme, the casein acts as a blocking solution to bind nonspecific sites on the plastic wells of the microtiter plate. Compared with the boiled casein solution, other blocking solutions such as powdered milk, gelatin, and BSA were less effective due to high background absorbance values. Although the indirect ELISA does not require blocking solutions, because the antibody coating the microtiter plate well captures the host-specific antigen in the bloodmeal sample (Service et al. 1986), the indirect and the direct ELISA have equal specificity for human and domestic hosts.

Table 2. Direct ELISA tests of seven host systems to compare specificity for host sera (1/500 in PBS) when conjugates were used alone or with heterologous serum

Host sera	*Host conjugate*[a]															
	Human		Cow		Chicken		Pig		Horse		Goat		Dog			
	A	S	A	S	A	S	A	S	A	S	A	S	A	S		
Human	1.762	1.622	0.094	0.086	0.090	0.023[b]	0.385	0.053[b]	0.652	0.052[b]	0.572	0.028[b]	0.670	0.044[b]		
Cow	0.063	0.042[b]	1.843	1.787	0.140	0.029[b]	0.307	0.054[b]	0.453	0.093[b]	1.775	0.054[b]	0.384	0.040[b]		
Chicken	0.049	0.047	0.094	0.086[b]	>2	>2	0.107	0.056[b]	0.049	0.058[b]	0.057	0.028[b]	0.131	0.036[b]		
Pig	0.118	0.034[b]	0.093	0.080[b]	0.127	0.026[b]	>2	1.340[b]	0.585	0.060[b]	0.715	0.031[b]	0.724	0.040[b]		
Horse	0.146	0.032[b]	0.077	0.074	0.211	0.012[b]	0.355	0.033[b]	>2	>2	0.785	0.019[b]	0.391	0.020[b]		
Goat	0.056	0.034[b]	0.081	0.076	0.136	0.026[b]	0.199	0.039[b]	0.303	0.056[b]	>2	0.619[b]	0.163[b]	0.031[b]		
Dog	0.225	0.045[b]	0.099	0.081[b]	0.084	0.026[b]	0.403	0.053[b]	1.329	0.103[b]	0.349	0.030	>2	>2		
Cat	0.130	0.038[b]	0.082	0.075	0.067	0.025[b]	0.495	0.49[b]	0.524	0.066[b]	0.493	0.029[b]	1.572	0.053[b]		
PBS	0.037	0.030	0.041	0.040	0.024	0.020	0.043	0.046	0.035	0.032	0.036	0.016	0.040	0.024		

[a] Values represent absorbance readings (range, 0–2) 30 min after the addition of substrate. A, conjugates used alone; S, conjugates used with heterologous serum (1:500 dilutions of each serum).

[b] $P < 0.05$, two-tailed t test.

Table 3. Bloodmeal determinations by direct ELISA for *A. gambiae* s. lat. and *A. funestus* collected inside huts from two sites in western Kenya

Host[a]	Saradidi				Kisian			
	A. gambiae		*A. funestus*		*A. gambiae*		*A. funestus*	
	No.	%	No.	%	No	%	No.	%
Human	404	82.0	118	86.7	1,909	88.2	1,410	91.3
Bovine	46	9.3	2	1.5	122	5.6	11	0.7
Human and bovine	13	2.6	3	2.2	40	1.9	17	1.1
Dog	0	0	0	0	4	0.2	0	0
Goat	0	0	0	0	6	0.3	0	0
Unknown	30	6.1	13	9.6	83	3.8	107	6.9
Total	493		136		2,164		1,545	

[a] Specimens not reacting with human or bovine were tested for dog, goat, pig, horse, and chicken blood meals.

The two-step ELISA employing peroxidase and phosphatase-labeled antihost immunoglobulins was developed for situations requiring additional information on a second, nonhuman host. The advantage is that reactions for the second host are obtained immediately after washing plates and adding the second substrate. The same procedure also worked well on bloodmeal samples for anophelines from Pakistan, where bovines were the predominant host. Procedures can be modified for use with most commercially available enzyme-labeled host immunoglobulins.

The direct ELISA proved useful for identifying blood meals of *A. gambiae* s. lat. and *A. funestus* collected inside houses in western Kenya. The high frequencies of human feeding, and secondarily of cattle feeding, by these species was expected based on previous studies in the Kisumu area (Joshi et al. 1975, Service et al. 1978, Highton et al. 1979). The two-step procedure, with antihuman peroxidase and antibovine phosphatase, enabled us to identify 94.4% of the blood meals in the first screening. Few blood meals of other domestic hosts were detected. Even though the ELISA tests screened for most available hosts in the rural Kenyan area, 5.4% (233/4,338) of the samples did not react. It is likely that most of these samples were processed after the bloodmeal IgG was digested, but some may represent other hosts.

In processing field-collected mosquitoes for bloodmeal determination, it is useful to record blood-feeding stages, which are temperature dependent and associated with ovarian development (World Health Organization 1975). The mosquitoes tested in Kenya were all freshly fed to ensure maximum host detection, but positive reactions were also obtained for 13/13 half-gravid mosquitoes from Kenya in preliminary testing. This is similar to the detection limits of the indirect ELISA (Service et al. 1986). Field-collected specimens can be stored dried or frozen (up to 50 mosquitoes per 0.5-ml vial) for at least 8 mo without losing reactivity. The direct ELISA can also be used with specimens smeared onto filter paper and stored dry for over

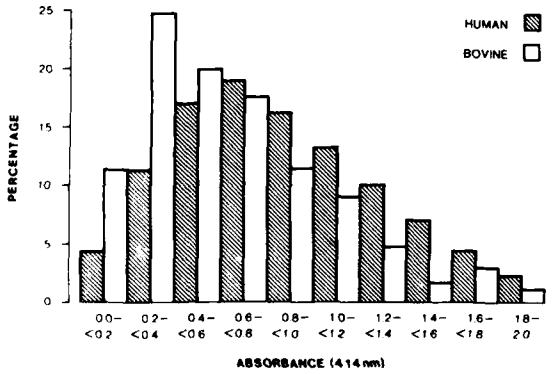

Fig. 3. Frequency distribution of ELISA absorbance values for identified human (n = 3,847) and cow (n = 181) blood meals of *Anopheles gambiae* s. lat. and *A. funestus* collected from two sites in western Kenya.

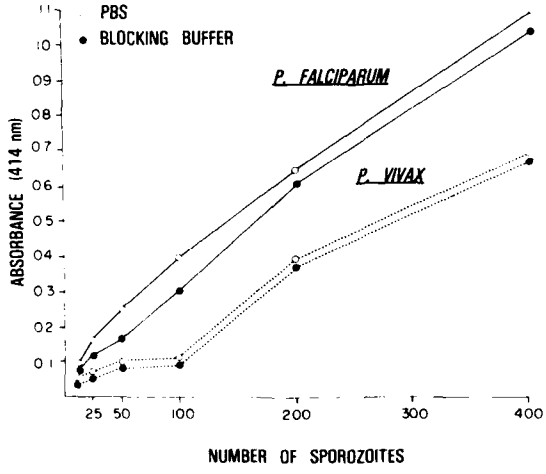

Fig. 4. Mean absorbance values for *Plasmodium falciparum* and *P. vivax* ELISA tests with blood-fed mosquitoes originally ground in 50 µl PBS (●) and mosquitoes ground in 50 µl blocking buffer (○), the solution used in the sporozoite ELISA. For mosquitoes originally ground in PBS, 10 µl was used for the bloodmeal ELISA and 40 µl was added to blocking buffer. Counted sporozoites were added to each sample (three mosquitoes per group) and dilutions ranging from 12 to 400 sporozoites per 50-µl sample were tested by the sporozoite ELISA's.

6 mo. Dried or frozen specimens may be preferable to filter paper samples, because the method of expressing the blood onto filter paper can affect the outcome of precipitin tests (Eligh 1952). Edman & Schmid (1970) have reviewed specimen storage procedures for precipitin tests, which should also be applicable to ELISA. Specimens can be stored at −20 to −70°C in PBS and tested when enough samples are accumulated.

For malaria field studies, the direct ELISA for blood meals can be used in conjunction with sporozoite ELISAs to determine rates of host feeding and mosquito infection rates. We have described a procedure for grinding whole mosquitoes in PBS and using aliquots for both tests. This may be practical only when sporozoite rates are less than 1% because ELISA detection of circumsporozoite antigen from oöcyst infections can yield inflated sporozoite rates (Beier et al. 1987). When infection rates are greater than 1%, it would be advisable to cut mosquitoes at the midthorax, or the juncture of the thorax and abdomen, and test abdomens only for bloodmeal identification.

The direct ELISA for bloodmeal identification should facilitate routine testing in most medical entomology laboratories. Although this assay was developed for mosquitoes, it also works for sand flies (P. G. Lawyer, personal communication) and should be suitable for all hematophagous insects. The assay should initially be standardized, following procedures outlined in this paper, because of the inherent variability of enzyme-labeled host immunoglobulins from different manufacturers and among lots from the same manufacturer. The ELISA should be tested against heterologous sera from different hosts in the study areas to determine cross-reactivity and appropriate dilutions of sera required in the conjugate step to increase specificity. The direct ELISA can be adapted for use in many geographic areas, provided appropriate controls are run and the limitations of the test are clearly determined.

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